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Positive Selection and Transplantation of Autologous Highly Purified CD133$^+$ Stem Cells in Resistant/Relapsed Chronic Lymphocytic Leukemia Patients Results in Rapid Hematopoietic Reconstitution without an Adequate Leukemic Cell Purging

Alessandro Isidori,[1,2] Maria Rosa Motta,[1] Monica Tani,[1] Carolina Terragna,[1] Pierluigi Zinzani,[1] Antonio Curti,[1] Simonetta Rizzi,[1] Simona Taioli,[1] Valeria Giudice,[3] Alessandra D'Addio,[1] Gabriele Gugliotta,[1] Roberto Conte,[3] Michele Baccarani,[1] Roberto M. Lemoli[1]

[1]Institute of Hematology and Medical Oncology, "L. & A. Seràgnoli," University of Bologna, Bologna, Italy; [2]Hematology and Bone Marrow Transplant Center, San Salvatore Hospital, Pesaro, Italy; [3]Immunohematology Service and Blood Bank, S. Orsola-Malpighi Hospital, Bologna, Italy

Correspondence and reprint requests: Alessandro Isidori, MD, Hematology and Bone Marrow Transplant Center, San Salvatore Hospital, Via Lombroso, 61100 Pesaro, Italy (e-mail: a.isidori@ospedalesansalvatore.it).

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ABSTRACT

We assessed the capacity of positively selected autologous CD133$^+$ hematopoietic stem cells (HSCs) to reconstitute lymphomyelopoiesis in chronic lymphocytic leukemia (CLL) patients receiving myeloablative chemotherapy. Ten resistant/relapsed CLL patients underwent HSC mobilization with chemotherapy and granulocyte-colony stimulating factor (G-CSF). Positive selection of circulating CD133$^+$ HSCs was performed by immunomagnetic technique. Highly purified HSCs were reinfused after busulphan/melphalan myeloablative treatment. A median number of 4.2 × 10^6 CD34$^+$ cells/kg and of 3.14 × 10^6 CD133$^+$ cells/kg were collected. Immunomagnetic selection resulted in the reinfusion of a median number of 2.45 × 10^6 CD133$^+$ cells/kg (median purity: 94.8%; median recovery: 84%) and 2.4 × 10^6 CD34$^+$ cells/kg (median purity: 93%; median recovery: 71%). HSC selection resulted in a median T cell and CD19$^+$/CD5$^+$ cell depletion of 3.85 log and 2.8 log, respectively. At the molecular level, however, 7 of 8 valuable purified HSC fractions were contaminated by leukemic cells. All CLL patients showed rapid and sustained myeloid engraftment after reinfusion of purified CD133$^+$ cells. Immunologic reconstitution was comparable to that routinely observed in patients reinfused with unmanipulated leukapheresis products and no late infectious complications were observed. With a median follow-up of 28 months for transplanted patients, 5 patients are in clinical complete remission, 3 are in partial remission, and 1 is in progression. In conclusion, the reinfusion of highly purified CD133$^+$ HSCs allowed the rapid and sustained recovery of hematopoiesis after myeloablative treatment in resistant/relapsed CLL patients. However, the purging potential of positive selection of CD133$^+$ cells is not adequate to achieve tumor-free autografts.

KEY WORDS

CD34$^+$ cells • CD133$^+$ cells • Positive selection • Purging • Autologous stem cell transplantation • Chronic lymphocytic leukemia

INTRODUCTION

CD133 is a novel antigen expressed on hematopoietic stem cells (HSCs). CD 133$^+$ cells are present in the peripheral blood (PB) of mobilized and nonmobilized individuals, in the bone marrow (BM), in cord blood (CB), and are capable of long-term reconstitution of human hematopoiesis in immunodeficient mice [1-3]. The expression of the CD133 antigen on HSCs is largely superimposable to that of CD34. CD133$^+$ cells can be cultured in vitro as well as CD34$^+$ or c-kit$^+$ populations [4,5], thereby suggesting that CD133$^+$ cells

share similar growth factor requirements. However, along with their hematopoietic potential, CD133$^+$ HSCs give origin to the endothelial lineage and are known to contribute to neoangiogenesis after tissue ischemia and organ regeneration in animal models [6-8]. Moreover, CD133 antigen is expressed on selected embryonic stem cell lines [9], human fetal neural stem cells with the potential to repair damaged neural tissue [10], nonhematopoietic adherent cells [11], and multipotent adult progenitor cells (MAPc), which can differentiate in vitro and in vivo, at the single cell level, into embryonic stem cell-like cells with visceral mesoderm, neuroectoderm, and endoderm characteristics [12]. Taken together, these studies clearly indicate that CD133 represents a cell surface marker for the identification of pluripotent human stem cells showing, perhaps, a wider differentiation potential than CD34$^+$ cells.

The potential clinical benefit of stem cell purification by positive selection of CD133$^+$ cells in comparison with CD34$^+$ cells is debatable [13-15]. Very few studies have recently evaluated the infusion of allogeneic or autologous CD133$^+$ cells to support the recovery of BM function after myeloablative chemotherapy [13-15]. Although conclusive evidence is still lacking, stem cell purification by CD133$^+$ expression to reduce tumor cell contamination in autografts may be considered for those disease(s) expressing CD34 but not CD133 [15].

Chronic lymphocytic leukemia (CLL) is characterized by the clonal proliferation and accumulation of neoplastic B-lymphocytes (and much more rarely T cells) in the blood, BM, lymph nodes, and spleen [16]. At present, there is no conventional curative treatment for CLL. In addition, in the presence of poor risk factors the median survival is short [17]. The use of new drugs, such as fludarabine, produced a high number of partial (PR) or complete (CR) clinical and molecular remissions, especially in previously untreated patients, but only a marginal improvement of overall survival (OS) [18]. Recently, there has been considerable interest for antibody-based therapeutic approaches [19-21], and their use resulted in excellent responses in some patients, although the follow-up is still short.

High-dose chemoradiotherapy followed by either autologous or allogeneic stem cell transplantation (SCT) further improves the remission rate. Conventional allogeneic SCT for CLL is associated with significant morbidity and mortality, both from regimen-related toxicity as well as from graft-versus-host disease (GVHD) and infection. Retrospective data reported from the European Bone Marrow Transplantation Registry (EBMTR) showed a high number of clinical and molecular remissions with a 3-year disease-free survival (DFS) of 40% [22]. However, treatment-related mortality (TRM) following allogeneic SCT in CLL patients was 46%, the mortality from GVHD being 20% [22]. A major advance in reducing the short-term morbidity and mortality of SCT has been the use of nonmyeloablative or reduced-intensity conditioning regimens [23-25]. RIC regimens appear to be associated with a decreased mortality after allogeneic transplantation, and allow transplantation in older patients, making this approach applicable to increased numbers of CLL patients [23-25]. Patients in these studies were often heavily pretreated and refractory to therapy. Despite these poor prognostic features, the majority of CLL patients demonstrated donor engraftment and high CR rate [23,24], although survival was improved only in patients with chemosensitive disease. Taken together, these studies provided perhaps the strongest direct evidence to date for a graft-versus-leukemia effect that can be exploited in the management of CLL.

A major drawback of allogeneic SCT is the limited number of CLL patients with an HLA-identical donor [26-28]. On the contrary, autologous stem cell transplantation (ASCT) is applicable for almost every patient without important comorbidity under the age of 70 years, and induces complete responses in some patients [26-31]. Although several studies have addressed the role of ASCT in the management of younger patients with CLL [29-31], its use remains controversial, with the major problem being disease relapse. In 1 study, on long-term follow-up of 137 patients who underwent autologous SCT with chemosensitive disease, progression-free survival (PFS) at 6 years was 30% (±4%), with no evidence of a plateau [26].

Multiparameter flow cytometric and molecular analyses are used to investigate whether persistence of minimal residual disease will predict clinical relapse following transplant in CLL. Moreover, there are concerns about the ability of CLL patients to mobilize adequate numbers of progenitor cells, particularly after fludarabine therapy [26]. Furthermore, a major drawback for the outcome of autologous transplantation in CLL is the high frequency of malignant cell contamination of BM and PB, which may contribute to relapse after transplantation. This finding has prompted investigations to develop effective purging techniques, mainly based on positive and/or negative immunoselection [30,31]. However, the existence of CLL cells expressing the CD34 antigen cannot be excluded, and such "tumor stem cells" might be enriched by purification according to CD34 expression [30].

On this rationale, we planned a pilot study for resistant/relapsed CLL patients to assess the capacity of positively selected autologous CD133$^+$ HSCs to reconstitute lymphomyelopoiesis after myeloablative chemotherapy.

Table 1. *Patient Characteristics*

Number of patients	**12**
Sex	
Male	**10**
Female	**2**
Median age (range)	**54 (46-62)**
Median body weight (range)	**73 (48-109)**
Rai Stage at diagnosis	
0	**—**
I	**—**
II	**4**
III	**7**
IV	**1**
Median diagnosis-ASCT interval in months (range)	**27 (10-138)**
Disease status	
Partial response	**10**
Refractory	**2**
Median lines of prior chemotherapy (range)	**2 (1-3)**
Patients receiving fludarabine before entering the study (%)	**100**
Median number of prior cycles with fludarabine (range)	**4 (2-6)**
Median time (months) from last cycle of fludarabine and stem cell mobilization (range)	**5 (1-41)**
Median extent of marrow infiltration at time of study entry (range)	**60 (10-90%)**

PATIENTS AND METHODS

Patient Population

Twelve resistant/relapsed CLL patients were enrolled between February 2003 and September 2005. The characteristics of study patients are summarized in Table 1. Patients with a histologic CLL diagnosis according to the REAL/International Lymphoma Study Group Classification [32] were eligible if they were between 18 and 65, relapsed or resistant after at least 1 chemotherapy regimen, with adequate renal, cardiac, and pulmonary function, a Karnofsky score $\geq$70%, or a WHO score $\leq$1. Exclusion criteria were age $<$18 or $>$65 years, major organ dysfunction, myelosuppressive chemotherapy within the last 4 weeks, CD5 negativity, and uncontrolled organ infection. Patients with a positive test for human immunodeficiency virus (HIV) or any form of active hepatitis were also excluded, as well as pregnant or nursing females. All patients were previously treated with fludarabine-containing regimens (Table 1). None of the patients received Alemtuzumab before entering the study.

All subjects gave written informed consent before entering the study. The protocol was approved by the Institutional Ethics Committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Chemotherapy and Mobilizing Treatment

All patients received high-dose cyclophosphamide (7 g/m^2) as mobilizing regimen. Granulocyte colony stimulating-factor (G-CSF) (Filgrastim, Granulokine, Amgen Inc., Thousand Oaks, CA) at the dose of 5 μg/kg/day was administered as a subcutaneous injection starting from day 6 after chemotherapy until successful PBSC mobilization.

Leukaphereses, PBSC Processing, and Positive Selection of CD133$^+$ HSCs

Stem cell collection was started when the absolute number of circulating CD34$^+$ and CD133$^+$ cells was $>$20 cells/μL [33-35] during the recovery phase from high-dose cyclophosphamide chemotherapy. PBSC were collected as already reported [33-35]. Standard volume leukaphereses were performed daily until the collection of a minimum cell dose of 2×10^6 CD133$^+$ cells/kg was achieved; 1×10^6 CD133$^+$ cells/kg was considered the minimum number of HSCs to proceed to transplant. CD133$^+$ cells were selected with the CliniMACS® device and anti-CD133 monoclonal antibody (MoAb) directly coupled to magnetic beads (CD133 Reagent, Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions. Briefly, CD133$^+$ cells were labeled with CD133 Reagent, washed, and subsequently purified using CliniMACS® technology. After CD133 selection, cells present in the positive and negative fractions were counted and submitted to flow cytometry analysis (see later). For exclusion of dead cells, 7-amino actinomycin D staining (PharMingen, San Diego, CA) was included in all fractions. Cryopreservation and storage in liquid nitrogen were performed by diluting CD133$^+$ cells in autologous plasma and 10% DMSO. The cell suspension was cooled in a programmed biologic freezing unit (CRYO 10-16, Planer, USA) at 1°C/min to $-$40°C, then at 5°C/min to $-$100°C and stored in the gas phase of a liquid nitrogen tank until thawing [33]. At the time of reinfusion, HSCs were rapidly thawed at 37°C and reinfused via a central line [33].

Conditioning Regimen, Supportive Care, and Clinical Monitoring

All patients were conditioned with oral busulphan 16 mg/kg/day over 4 days (days $-$6 to $-$2) and intravenous melphalan 140 mg/m^2/day for 1 day (day $-$2) before reinfusion of CD133$^+$ autologous stem cells. All patients received 5 μg/kg/day of filgrastim (Amgen) starting on day +6 from autograft. Patients were nursed in single or double rooms in reverse isolation and received antimicrobial prophylaxis that consisted of oral nystatin and ciprofloxacin. Packed red blood cells and platelet transfusions were administered to maintain a hemoglobin level $>$8 g/dL and a platelet count $>10 \times 10^9$/L. Patients were treated with broad-spectrum antibiotics when fever developed and the ANC was $<0.5 \times 10^9$/L. Amphotericin B (1 mg/kg/day) was added if patients had persistent fever after 4-7

days of intravenous antimicrobial therapy. Patients underwent daily assessment of symptoms and physical examination during hospitalization and weekly after discharge. As previously reported [34], laboratory workup was performed before transplant, daily during the hospital stay, and weekly after discharge. Cotrimoxazole and acyclovir were administered to CLL patients for 3 months after ASCT to prevent *Pneumocystis carinii* and viral infections, respectively.

Immunophenotypical Analysis

Flow cytometry analysis was performed by conventional dual color immunofluorescence using a panel of fluorescein isothiocyanate (FITC)-conjugated and phycoerythrin (PE)-conjugated monoclonal antibodies (mAbs): 8G12-PE (HPCA2/CD34) anti-CD34, HLE-1-FITC (CD45), Leu-12-FITC (CD19), and Leu-1-PE (CD5) (Becton Dickinson, San Jose, CA). PE-conjugated anti-CD133 was purchased from Miltenyi Biotec. Negative controls were isotype-matched irrelevant mAbs (Becton Dickinson). Briefly, 100 μL of PB in heparin-containing tubes (Becton Dickinson) were incubated for 15 minutes at room temperature with 10 μL of mAbs. Red cell lysis (FACS™ Lysing Solution, Becton Dickinson) was performed when appropriate. The samples were then centrifuged, washed twice with phosphate buffer, and fixed with 1% paraformaldehyde (Sigma, St. Louis, MO). Cells (1×10^5) were acquired by flow cytometer (FACSCalibur; Becton Dickinson) and analyzed by CellQuest software (Becton Dickinson) [32-35]. The analysis was performed excluding cellular debris in a SSC/FSC dot plot. The percentage of positive cells was calculated subtracting the value of the appropriate isotype controls. The sensitivity for detection of CD19$^+$ CD5$^+$ CLL cells by FACS was 1 tumor cell in 10^4 normal cells, as shown in preliminary experiments with mixtures of CLL cells and leukapheresis products from normal donors (data not shown).

Molecular Analysis

Nucleic acid extraction, cDNA synthesis, identification of V-D-J gene rearrangement, and detection of contaminating CLL cells were performed as previously reported [36]. BM samples, samples of unmanipulated leukapheresis, and highly purified HSC populations were analyzed at diagnosis, before and after positive selection of CD133$^+$ cells, and at regular time points after transplant. No false-positive results were obtained when the RNA from other CLL patients was used for negative cross controls. The sensitivity of the MRD detection ranged from 1 tumor cell in 10^4 to 10^7 cells (median 1 cell in 10^5).

Clinical Endpoints

The primary endpoints of the study were: (1) to assess the capacity of positively selected CD133$^+$ cells to reconstitute autologous hematopoiesis of CLL patients undergoing myeloablative chemotherapy, and (2) to assess the efficacy of positive selection of CD133$^+$ cells to remove neoplastic CLL cells from autologous grafts (indirect purging). Secondary endpoints were: (1) to determine the immunologic reconstitution of CLL patients receiving myeloablative chemotherapy and reinfusion of CD133$^+$ cells; (2) to assess the molecular complete remission rate (MCR); and (3) to assess the safety of this procedure, considered as the incidence of adverse event (grading according to WHO) and clinically significant abnormal laboratory value following reinfusion of highly purified CD133$^+$ cells.

Statistical Analysis

The results are presented as median values and ranges. The correlation between the number of CD34$^+$ and CD133$^+$ cells infused and the time to hematopoietic recovery were assessed by the Pearson's correlation test.

RESULTS

Twelve CLL patients entered in the study. Two of 12 patients did not mobilize the minimum number of PBSC to proceed to transplantation procedures. Conversely, 10 patients underwent leukaphereses followed by positive selection of CD133$^+$ cells. Nine out of 10 patients were then transplanted, as 1 patient withdrew the informed consent to ASCT while in CR. Finally, only 8 of 9 patients undergoing ASCT were valuable for molecular analysis.

Cell Processing and Purging Results

A median number of 4.2×10^6 CD34$^+$ cells/kg (range: 2.75-8.62) and of 3.14×10^6 CD133$^+$ cells/kg (2.25-7.18) were collected from the PB after mobilization with high-dose cyclophosphamide and G-CSF. Immunomagnetic selection with CD133 reagents allowed the reinfusion of a median number of 2.45×10^6 CD133$^+$ cells/kg (1.51-6.41) with a median purity of 94.8% (76.5-99.5) and a median recovery of 83.5% (66.5-100), and 2.4×10^6 CD34$^+$ cells/kg (1.15-6.5) with a median purity of 93% (73.8-99.5) and a median recovery of 71% (45-82) (Table 2). Positive selection of CD133$^+$ cells resulted in a median T cell depletion of 3.85 log (1.6-4.5) and in the median purging of 2.8 log (1-4.5) of CD19$^+$/CD5$^+$ cells (Table 3). Each positive selection required only 1 column and 1 CD133$^+$ tubing set.

Table 2. *Median Number of CD34$^+$ and CD133$^+$ Cells before and after the Selection Procedure (=10 Patients)*

Median Values	Collected	Reinfused	Recovery (%)
CD34$^+$ cells			
×10^6/kg	4.2 (2.75-8.62)	2.4 (1.15-6.5)	71 (45-82.3)
%	0.85 (0.37-3.22)	93 (73.8-99.5)	
CD133$^+$cells			
×10^6/kg	3.14 (2.25-7.18)	2.45 (1.51-6.41)	83.5 (66.5-100)
%	0.66 (0.33-2.68)	94.8 (76.5-99.5)	

Myeloid Engraftment

Nine of 10 CLL patients were reinfused with highly purified CD133$^+$ HSCs, and their rapid myeloid engraftment is shown in Table 4. One patient in clinical CR before HSC collection refused ASCT. Consistent with the fast hematopoietic reconstitution, the percentage of documented infections was low and the hospital stay after ASCT was short (Table 4). No significant correlation was found between the number of CD133$^+$ cells reinfused and time to neutrophil and platelet engraftment (data not shown). The extra-hematologic toxicity of ASCT supported with positively selected CD133$^+$ HSCs was mild: 1 patient showed WHO grade III oral mucositis and 1 patient had grade IV nausea/vomiting. One additional patient was readmitted into the hospital after developing a grade II-III sinusoidal obstructive syndrome (SOS) 30 days after the busulphan-containing conditioning regimen. After appropriate medical therapy the patient fully recovered from the SOS and she is now alive and well at 21 months from ASCT.

Immunologic Reconstitution and Late Infectious Complications

Figures 1 and 2 show the immunologic reconstitution of CLL patients after transplantation of CD133$^+$ HSCs. The recovery of CD3$^+$, CD4$^+$, CD8$^+$ T lymphocytes and CD16$^+$/CD56$^+$ natural killer cells is reported in Figure 2, whereas Figure 1 shows white blood cell and B cell reconstitution. The total lymphocyte count as well as the number of CD3$^+$, CD8$^+$, and natural killer cells returned to baseline value by 1 month after transplantation. As expected, the median value of CD4$^+$ cells remained below 0.5 × 10^9/L up to 18 months after ASCT, and became higher than baseline levels at 2 years after ASCT (Fig. 2). Clinically, no infectious complications were observed and none of the patients were readmitted in the Hospital for late infections.

Table 4. *Engraftment Data (=9 Patients)*

	Median	Range
Days to ANC >0.5 × 10^9/L	11	10-16
Days to PLT > 20 × 10^9/L	12	11-18
Days to PLT > 50 × 10^9/L	31	15-75
Documented infections (% of patients)	33	—
Parenteral antibiotics days	4	0-6
Days on G-CSF after ASCT*	8	6-13
Days to hospital discharge	14	12-19

ANC indicates absolute neutrophil count; PLT, platelet count; ASCT, autologous stem cell transplantation; G-CSF, granulocyte-colony stimulating factor.

*All patients received 5 μg/kg/day of filgrastim starting on day +6 after autograft.

Clinical and Molecular Follow-up

All mobilizing patients (=10) are evaluable for clinical follow-up, and 8 of 10 for molecular follow-up. With a median follow-up of 28 months for transplanted patients, 5 patients are in CR, 3 are in PR, and 1 is in progression. Three patients improved the clinical status after ASCT from PR to CR. At the molecular level, 7 of 8 valuable patients were reinfused with purified HSC populations still containing detectable leukemic cells (Table 5). Evaluation of molecular minimal residual disease at 6 months after ASCT showed the persistence of CLL cells in all evaluable patients.

DISCUSSION

CLL remains an incurable disease and, notwithstanding the excellent remission rates now achieved with purine analogs and mAbs, the vast majority of patients with CLL relapse after primary treatment [26]. With many potential treatments available, the sequence of treatments and the timing of procedures such as SCT remain controversial. Myeloablative allogeneic SCT is associated with increased morbidity and mortality. Although no direct comparisons of myeloablative and nonmyeloablative transplants have been performed for patients with CLL, given their

Table 3. *Median Number of CD3$^+$ and CD5/19$^+$ Cells before and after the Selection Procedure (=10 Patients)*

Median Values	Collected	Reinfused	Purging (log)
CD3$^+$cells			
×10^4/kg	1710 (957-5038)	0.56 (0.09-15.2)	3.85 (1.6-4.5)
%	4.18 (1.1-19.8)	0.29 (0.02-5.8)	
CD5$^+$/CD19$^+$cells			
×10^4/kg	813.5 (0-79,927)	1.7 (0-4.8)	2.8 (1-4.5)
%	1 (0-91.8)	0.26 (0-1.54)	

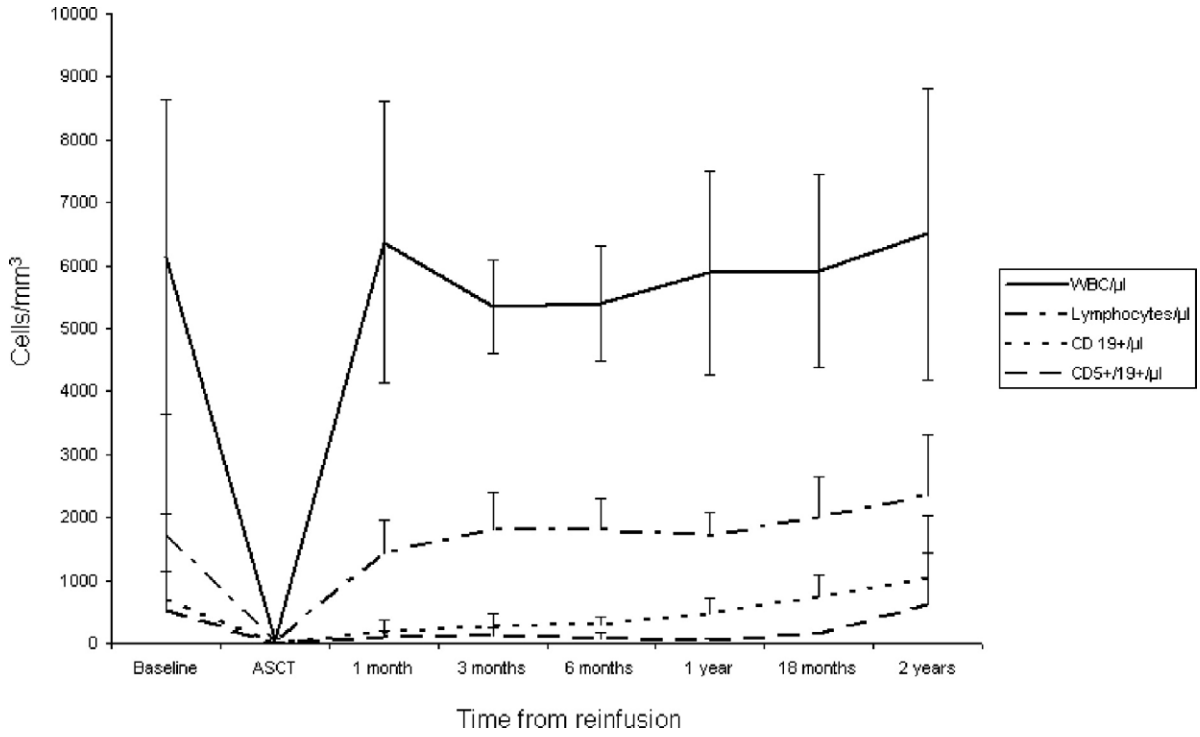

Figure 1. Immunologic reconstitution of normal and leukemic B lymphocytes in CLL patients after transplantation of highly purified autologous CD133+ HSCs.

older age, it seems most reasonable to consider nonmyeloablative conditioning regimen transplants as the approach of choice for CLL patients in whom allogeneic transplant is being considered. Several prospective multicenter trials [27-30] have addressed the role of ASCT in the management of patients with CLL. Its use still remains controversial, with a significant decrease in the number of these procedures after the

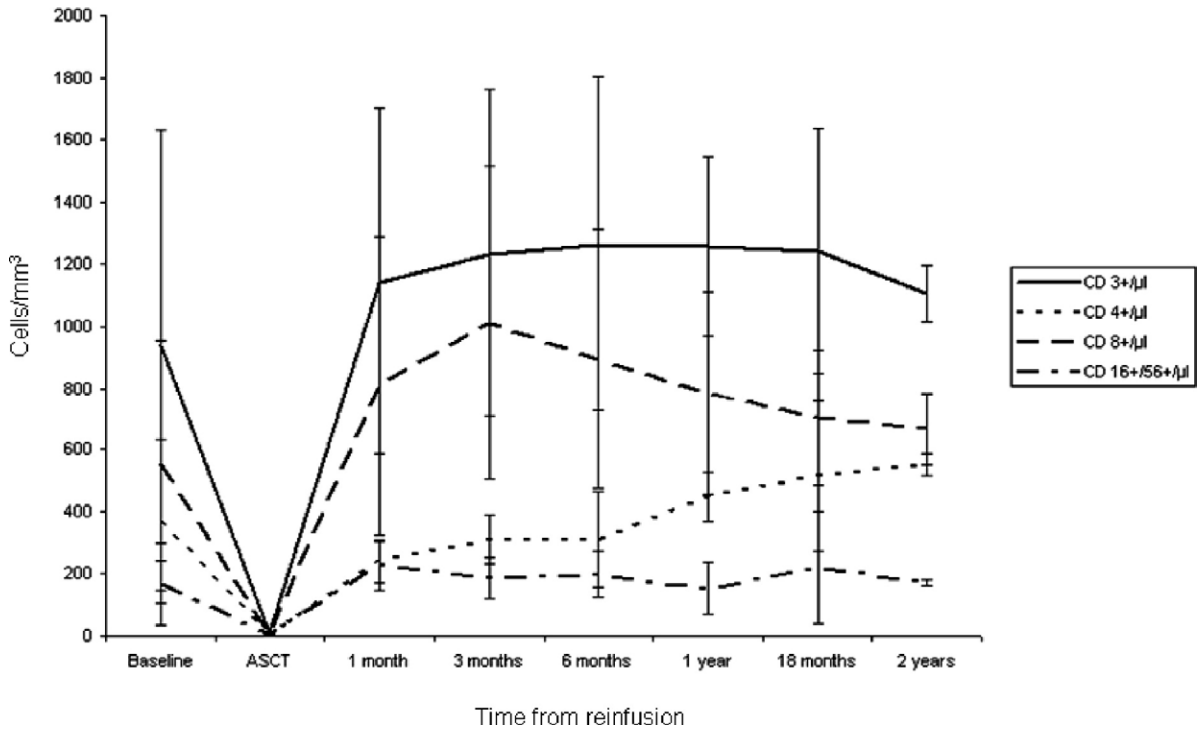

Figure 2. Immunologic reconstitution of T lymphocytes and NK cells in CLL patients after transplantation of highly purified autologous CD133+ HSCs.

Table 5. *Molecular Follow-up of CLL Patients Submitted to ASCT with Positively Selected CD133$^+$ HSCs*

Patient	Status at Study Entry In Vivo	Leukapheresis	Positively Selected CD133$^+$ HSCs	Follow-up (6 Months after ASCT)
1	+	+	+	+
2	+	−	−	+
3	+	−	+	+
4	+	+	ND	ND
5	+	+	+	+
6	NE	NE	NE	NE
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+

+ indicates PCR positive; −, PCR negative; NE, not evaluable; ND, not done.

introduction of RIC transplants and chemoimmunotherapy strategies.

Some studies demonstrated that high-dose therapy followed by autologous stem cell rescue can induce or maintain long-term complete remission [27-30]. However, disease relapse almost inevitably occurs after ASCT in CLL patients with the potential contribution of reinfused tumor cells. Immunomagnetic selection of CD34$^+$ cells is widely considered one of the most efficient methods of depletion of unwanted cells from PBSC grafts, resulting, for example, in B cells reduction of about 3 logs [33-35,37-38]. However, probably because of the high contamination of leukemic cells prior to purging procedures, most PBSC grafts from CLL patients contain residual tumor cells after conventional CD34$^+$ cell selection [30-31]. In addition, the existence of CLL cells expressing the CD34 antigen cannot be excluded; such "tumor stem cells" might be enriched by purification according to CD34 expression [30]. In fact, at least 2 studies addressed the question of the involvement of CD34$^+$ cells as a part of the malignant cell clone of patients with B-CLL [39-40]. In an elegant experimental model, Gahn and collaborators [39] tested BM and PB samples from 75 patients with B-CLL for the presence of trisomy 12 or the deletion of the retinoblastoma (Rb) gene by fluorescence in situ hybridization. CD34$^+$ subpopulations were isolated by fluorescence-activated cell sorting and analyzed for the presence of the informative genetic marker. Trisomy 12, as well as Rb deletion, was detected in 20% of patients. In 7 patients with trisomy 12, hematopoietic progenitor cells were sorted, and the genetic marker was detected in the CD34$^+$/CD38$^+$ cells as well as in the CD34$^+$/38$^-$ subsets in 3 patients. Progenitor cells were also sorted in 2 patients with Rb deletion. In 1 patient, Rb deletion was present in 10% of CD34$^+$/38$^+$ cells of only 1 patient. The authors concluded that the malignant transformation in B-CLL may involve early HSCs, and place a note of caution on strategies using ASCT. Although the expression of the CD133 Ag on HSCs is largely superimposable to that of CD34, there is no evidence, at present, of the potential involvement of CD133 in the B-CLL neoplastic clone. In this view, preliminary experiments from our laboratory did not show the coexpression of CD133 on CD19$^+$/CD5$^+$ leukemic cells (data not shown).

Based on these considerations, we designed a pilot study: (1) to assess the capacity of positively selected CD133$^+$ cells to reconstitute autologous hematopoiesis of CLL patients undergoing myeloablative chemotherapy, and (2) to assess the efficacy of positive selection of CD133$^+$ cells to remove neoplastic CLL cells from autologous grafts (indirect purging).

The main objective of our study was to demonstrate the safety of autografting with highly purified autologous CD133$^+$ HSCs. The most important determinants of safety were considered: (1) engraftment, (2) incidence of unexpected severe adverse event, and (3) infectious complications.

All patients had a rapid and sustained engraftment, with a median time to achieve an ANC count of 0.5×10^9/L and a platelet count of 20×10^9/L similar to those expected from infusion of the same number of CD34$^+$ cells in an unmanipulated autograft [33-35,37,38]. No significant correlation was found between the number of CD133$^+$ cells reinfused and time to neutrophil and platelet engraftment.

Consistent with the fast hematopoietic reconstitution, the percentage of documented infections was low and the hospital stay after ASCT was short. After the transplant, all patients rapidly recovered a number of CD4$^+$ T lymphocytes >200/μL. None of the patients experienced a CMV reactivation nor other opportunistic infections after hospital discharge. The extrahematologic toxicity of ASCT supported with positively selected CD133$^+$ HSCs was mild: only 1 patient developed an unexpected severe adverse event (a grade II-III sinusoidal obstructive syndrome).

The tumor burden of most of the patients enrolled in this study was very high at the time of stem cell collection (median marrow infiltration, 60%; Table 1), which resulted in a high tumor cell load in the leukapheresis products prior to positive selection of CD133$^+$ cells. Thus, in our study, CD133$^+$ HSC selection resulted in a median CD19$^+$/CD5$^+$ depletion of 2.8 log with detectable tumor cells in 7 of 8 evaluable purified HSC fractions, whereas double (positive/negative) selection techniques described by several groups [30,31] were able to produce a purging efficacy of about 5 logs.

With a median follow-up of 28 months for transplanted patients, 5 patients are in clinical, but not molecular, CR, 3 are in PR, and 1 is in progression.

Three patients improved the clinical status after ASCT from PR to CR. The follow-up of our cohort of patients is very short, and therefore, no speculation can be made on the long-term impact of this strategy. Moreover, the study was neither designed nor powered to assess the clinical efficacy of this procedure to induce complete clinical and molecular remissions.

In conclusion, positive selection and transplantation of autologous highly purified CD133$^+$ stem cells in resistant/relapsed CLL patients resulted in rapid hematopoietic reconstitution without an adequate leukemic cell purging.

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